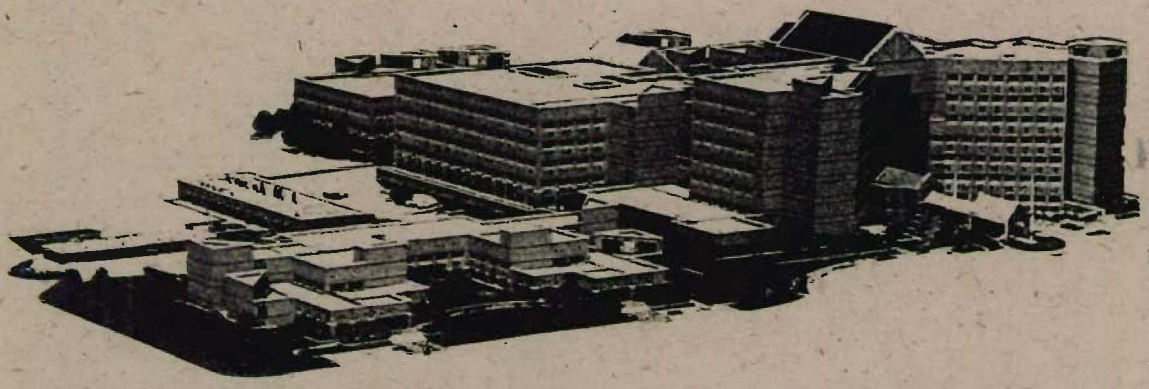
USAISR TECHNICAL REPORT 2005-06

Results of Topical Myeloperoxidase/Glucose Oxidase/Glucose in the Walker-Mason Burn Model

David G. Baer PhD and Joanna R. Reeder

December 2005



UNITED STATES ARMY INSTITUTE OF SURGICAL RESEARCH FORT SAM HOUSTON TEXAS

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14. ABSTRACT

One area of research at the ISR is soft tissue trauma encompassing infection control and wound healing. Treatments are evaluated in vivo using the Walker-Mason model which is a full thickness thermal injury and infection model. We have identified a novel proprietary topical antimicrobial which may be amenable to treating a variety of wounds. The system consists of an enzyme, myeloperoxidase (MPO) and the requisite substrates which have been optimized for the production of bactericidal reactive oxygen species. Sepsis data is gathered for 21 days and end survivorship of the test article groups are compared to the survivorship of the untreated group. The survival data is supplemented by monitoring the bacterial infection via bioluminescent imaging by using genetically engineered P. aeruginosa (ATCC 59-1244). The survivorship data is conclusive in stating the Topical Myeloperoxidase antimicrobial was unsuccessful in recovering an animal from septic infection. The imaging data shows the movement of bacteria from the dorsum to the internal cavity and infection of internal organs.

15. SUBJECT TERMS
Rat, Burn, Infection, Topical antimicrobial

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REPLY TO
ATTENTION OF

MCMR-SRZ

16 Nov 2005

MEMORANDUM FOR Commanding General, US Army Medical Research and Materiel Command (MCMR-ZA), 504 Scott Street, Ft. Detrick, MD 21702-5012

SUBJECT: Information Copy of Technical Report

1. The technical report titled "Results of Topical Myeloperoxidase/Glucose Oxidase/Glucose in the Walter-Mason Burn Model" by David Baer PhD has been reviewed by this Command and meets acceptable standards for publication. The report contains no matter that warrants disapproval for security or policy reasons.

2. The above technical report has been submitted to the Defense Technical Information Center (DTIC) and the ISR Library.

Encl

JOHN B. HOLCOMB
COL, MC
Commanding

CF:
Information Management Division (MCMR-CZ-I)

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REPLY TO
ATTENTION OF

MEMORANDUM FOR COMMANDER 9 Nov 2005

SUBJECT: Technical Report Review

1. I have reviewed the attached technical report and appropriate committee (IACUC/IRB) approval titled "Results of Topical Myeloperoxidase/Glucose Oxidase/Glucose in the Walker-Mason Burn Model" by David Baer PhD. This work represents the results of experiments conducted under:

Title of Protocol: Rat Excision Model of Wound Infection – A Type Protocol

Protocol Number: A-02-010-TP

Principal Investigator: David Baer, PhD

2. The technical report meets the requirements of good scientific merit.

3. I recommend that the attached technical report be approved for submission to DTIC and the ISR Library.

RECOMMENDATION:

APPROVAL/DISAPPROVAL

Cheryl D. DiCarlo

CHERYL D. DICARLO
LTC, VC
Director of Research

Results of Topical Myeloperoxidase/Glucose Oxidase/ Glucose in the Walker-Mason Burn Model

Introduction

At the ISR, research is centered on improving the medical treatment of combat casualties. One area of research is soft tissue trauma encompassing infection control and wound healing. Treatments are first evaluated *in vitro* and *ex vivo* and the final assessment is *in vivo* using two rat injury model. The two injury models used are full thickness thermal (Walker-Mason scald) injury and excision injury. While these models are similar in area injured, treatments, and data collection, they provide different but complementary tests of various treatments. The Walker-Mason model is designed to evaluate topical treatments that will decontaminate a wound and prevent sepsis. This model is used to screen various treatments including novel dressings and proprietary antimicrobial solutions. They are tested in attempt to find self-administrable treatments that are efficient and easy-to-use. As part of this program we have identified a novel proprietary topical antimicrobial which may be amenable to treating a variety of wounds. The system consists of an enzyme, myeloperoxidase (MPO) and the requisite substrates which have been optimized for the production of bactericidal reactive oxygen species.

The primary data to both models is comparative sepsis rate in the treatment groups to that of the untreated group. There are three control groups (untreated, injury only, and positive control). The expected result is to have near 100% mortality in the untreated group, near 100% survivorship in the injury only and the positive control; and the treatment groups'survivorship fall in between. The survival data is supplemented by monitoring the bacterial infection via bioluminescent imaging.

In order to study luminescence in a bacterial infection, *P. aeruginosa* (ATCC 59-1244) was genetically engineered to luminesce. The bacterial infection is monitored by non-invasive bioluminescent imaging as a means to assess the animals' infection chronologically.

Methods

All data presented were collected from animals used in IACUC approved protocols. All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory

Animals in an AAALAC approved facility. Surgical procedures are done using 2-3% Isofluorane via a facemask. Buprenorphine is administered at 0.1mg/kg twice a day for pain during the first two or three days of the study, and continued as needed.

Walker-Mason Scald Model

- Anesthetized rat is placed in a mold that exposes a 4 cm by 10 cm (20% body surface area) of dorsum.
- The dorsum is immersed in 100°C water for 10 seconds, producing a full thickness injury.
- The wound is inoculated with approximately 10^7 CFU *P. aeruginosa/ lux* in 1 ml of saline.
- The antimicrobial is applied in a controlled volume once a day for 10 days. The positive control, Silvadene (King Pharmaceuticals, Bristol, TN), is applied by evenly spreading on the wound area.

Sepsis data is gathered for 21 days. The end survivorship in the test article groups are compared to the survivorship of the untreated group using a two-sided Fisher Exact Test.

Animals are humanely euthanized at 21 days post injury or when an animal is determined to be morbid. Samples of the wound are collected for histology, biochemical assays, and organs were imaged to detect the presence of luminescent bacteria.

Test Articles

- Test articles obtained as High and Low Proprietary Antimicrobial Compounds (PAC);
    - High- 1 part activator to 2 parts 760 diluent
    - Low- .5 part activator to 2.5 parts 770 diluent
    - Made daily, used in 30 minutes time, peroxide monitored
    - 3 ml sprayed on using a Mucosal Atomization Device followed by swabbing with a sterile cotton tip applicator to ensure even distribution.

Non-Invasive Imaging

An imaging system and software (Xenogen, Alameda, CA) are utilized to non-invasively visualize bacteria *in vivo* during wound colonization and infection. This imaging minimizes the number of animals needed for the experiment as time course studies can be done on individual animals, and each animal serves as its own control. Rats are imaged non-invasively periodically for 21 days or until moribund. This provides a tool to monitor the progress of the infection. Septic animals are euthanized and a necropsy is performed with imaging of the organs to determine the distribution of bacteria in animals that succumbed infection.

Anesthetized animal is placed on a heated stage in a light tight box and an image is acquired using a highly sensitive CCD camera. Software produces a composite image by superimposing the luminescent image (that has pseudocolor added) over a white light image, and subtracts the background (Figure 1). Images are evaluated by chronologically compiling the images into mosaics that provide a spatial evaluation of active bacterial infection. This provides information as to the total area infected and invasiveness of infection.

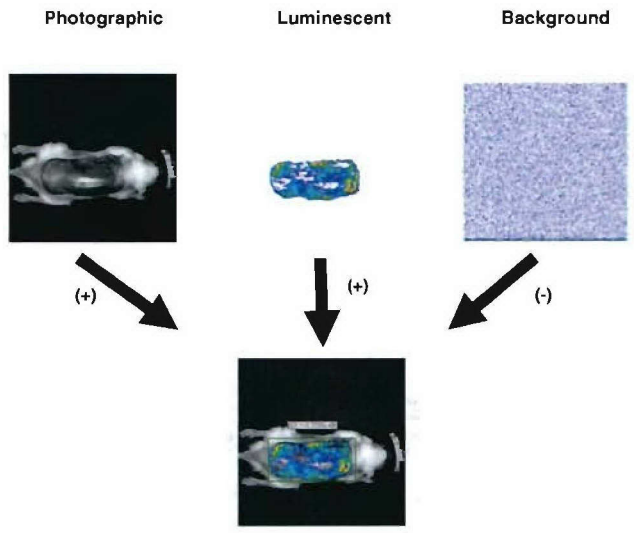


Figure 1. Luminescence imaging methodology.

Results

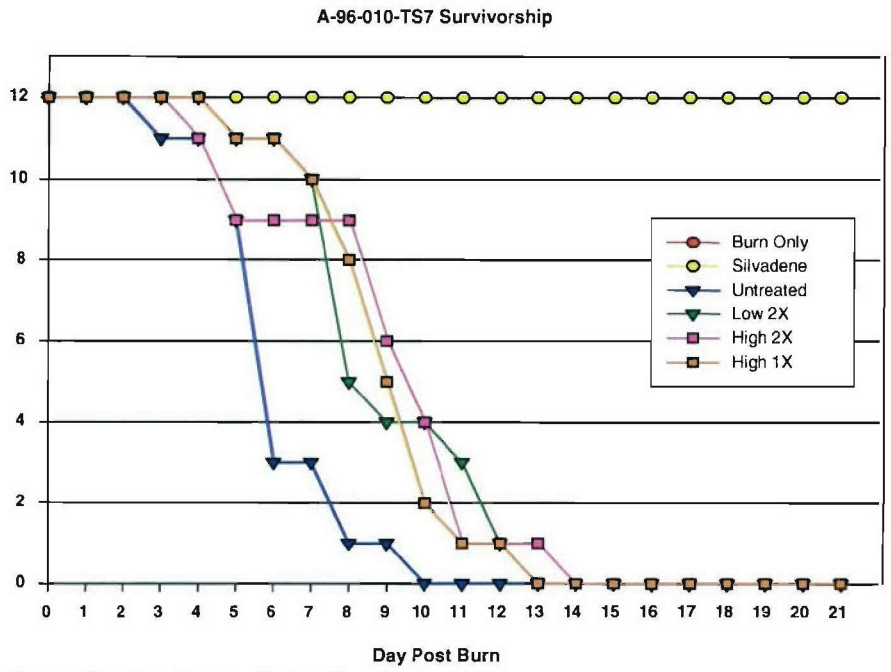


Figure 2. Number of animals surviving by day post burn.

	Survivorship	p value (compared to Untreated)
Burn Only	12/12	
Untreated	0/12	
Low 2X	0/12	N.S.
High 2X	0/12	N.S.
High 1X	0/12	N.S.
Silvadene	12/12	

Table 1. Number of animals surviving to post burn day 21 and p value computed by Fishers Exact Test using untreated animals for independent pairwise comparison to test groups

Low concentration twice a day, High concentration twice a day, and High concentration once a day are not significantly different from the Untreated group ($p>0.05$) (Figure 2 and Table 1). All quality control groups (Burn Only and Silvadene) performed as expected and within quality control limits.

Imaging

The image mosaic is one of four animals imaged in a group of twelve. All images were acquired before treatments. This technology is to aid in the understanding of the infection's progression and gives further insight into contamination and infection in experimental animals. A mosaic is

presented below (Figure 3) to aid in comparison of groups by selection of an example animal for each group. All of the available imaging data is included in Appendix A. In the Untreated, Low 2X, and H1X groups the animal has approximately 80% of the dorsum covered with bacteria. The Silvadene and H2X have foci of luminescence. Moving through time, those animals that become moribund and die have a progression of the luminescence outside the margin of the wound.

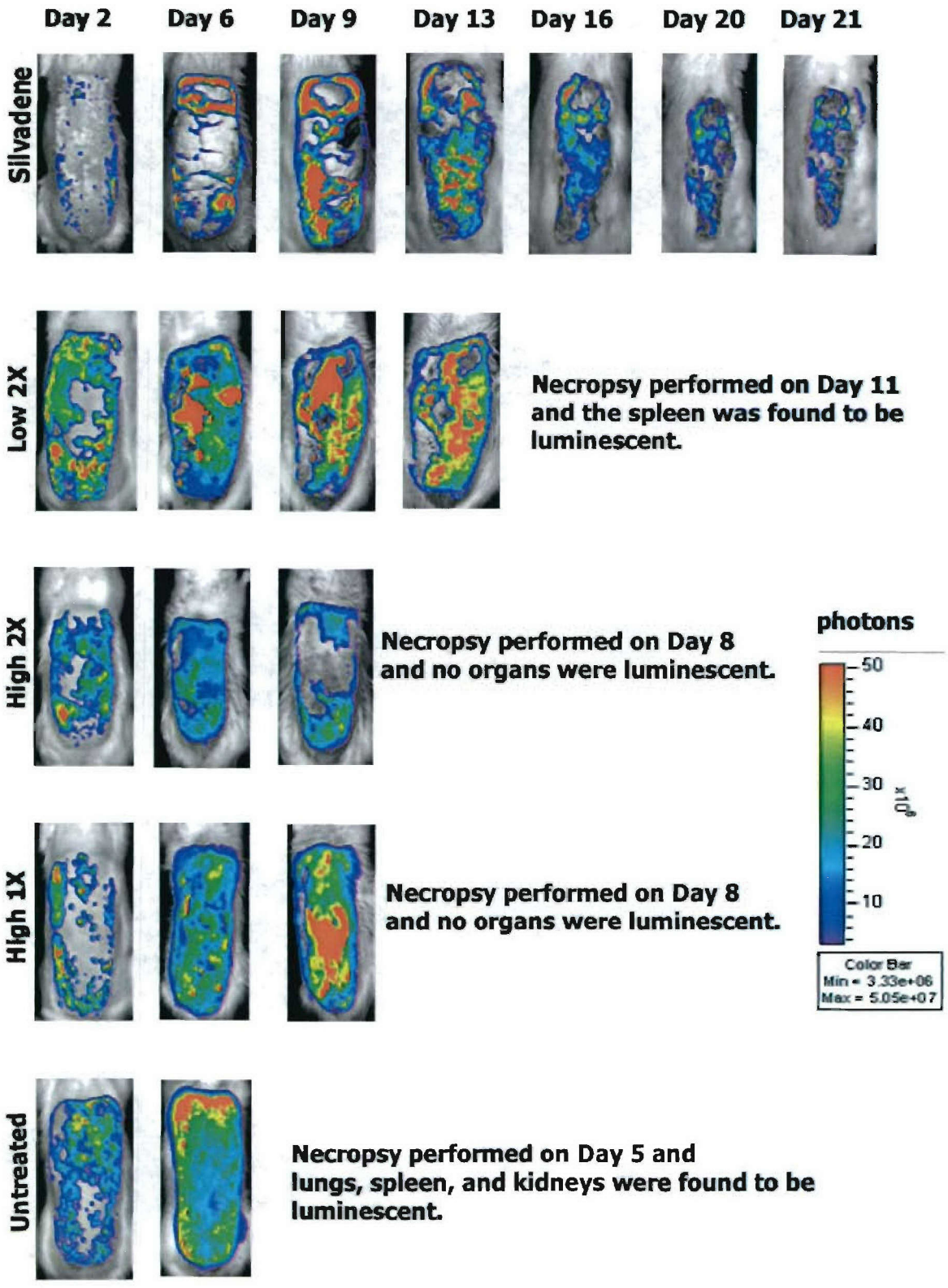


Figure 3. Composite image of a representative animal from each group for comparison of luminescence intensity

Conclusions

The Walker-Mason model of burn wound infection has a long history of use at the ISR in the evaluation of the pathophysiology of burn, the investigation of host/pathogen interaction, and the development and testing of antimicrobial agents. The quality control groups performed as expected based on experience with this model.

The animals that received a 20% BSA full thickness burn recovered uneventfully from this injury, weight loss ended by day 4, and animals reached their average pre-burn weight on day 9. Animals which were injured, inoculated and then not treated with antimicrobial lost weight continuously through the course of the experiment. On day 3, the first animal in this group was determined to be moribund and was euthanized; all animals in this group were euthanized by day 10. These results are typical for the Walker-Mason model.

The group which was injured, inoculated and treated with Silvadene had 0% mortality through the 21 day observation period. The average weight of animals in this group shows a pattern similar to that of the uninfected group, where initial weight loss ended by day 4, followed by continuous weight gain through the end of the experiment. Imaging of the luminescent bacteria showed that the bacteria took longer to colonize the eschar than in the untreated group, and that the luminescent signal did not reach as high levels as those seen in the untreated animals. Most importantly, contamination was contained to the necrotic tissue of the eschar and never spread to the surrounding tissues. These results are consistent with previous experience in this model.

The survivorship data is conclusive, the experimental treatments were not successful in preventing infection as there were no statistically significant differences between the survivorship in any of the experimental groups compared to the untreated controls. Average weight for each of these groups (Figure 4) showed a pattern that was similar to the untreated controls, where animals continued to loose weight through the course of the experiment. Between days 8 and 10 there was apparent average weight gain in some groups, however much of this can be attributed to the exclusion of the animals which had lost the most weight as these animals were euthanized.

When the images of all animals were inspected, the animals that had been treated twice a day with the highest concentration of the antimicrobial appeared to have improved bacterial contamination levels at day 6 and possibly day 2 as compared to untreated animals. In order to investigate this pattern, we graphed the group average for flux (a measure of the intensity of the luminescence) on post burn days 2 and 6 (Figure 5). This graph suggests that though the

treatments were not sufficient to prevent mortality, the high concentration solution delivered twice a day did have some positive impact on reducing contamination. Caveats to this analysis include the fact that only a subset of animals from each group were imaged, and this reduction in luminescence correlated with neither increased survival nor extended survival time.

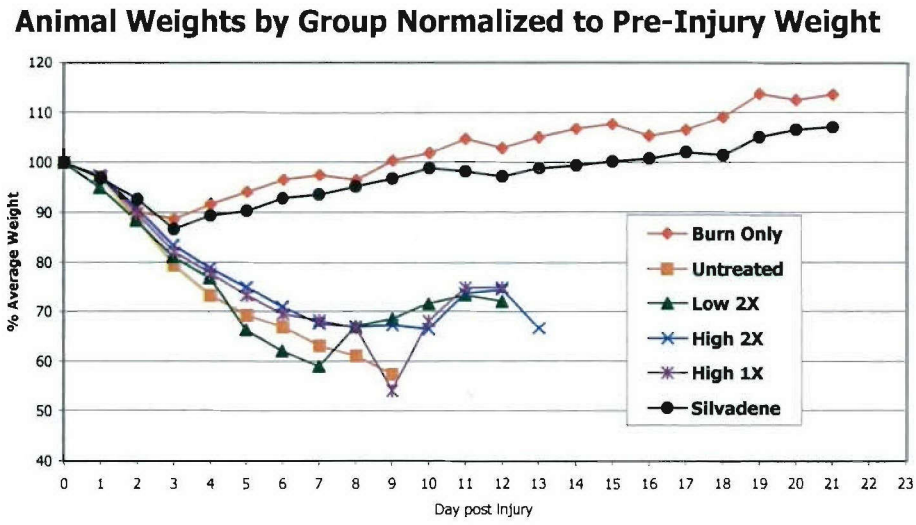


Figure 4. Average weight of each treatment group expressed as a percentage of pre-injury weight.

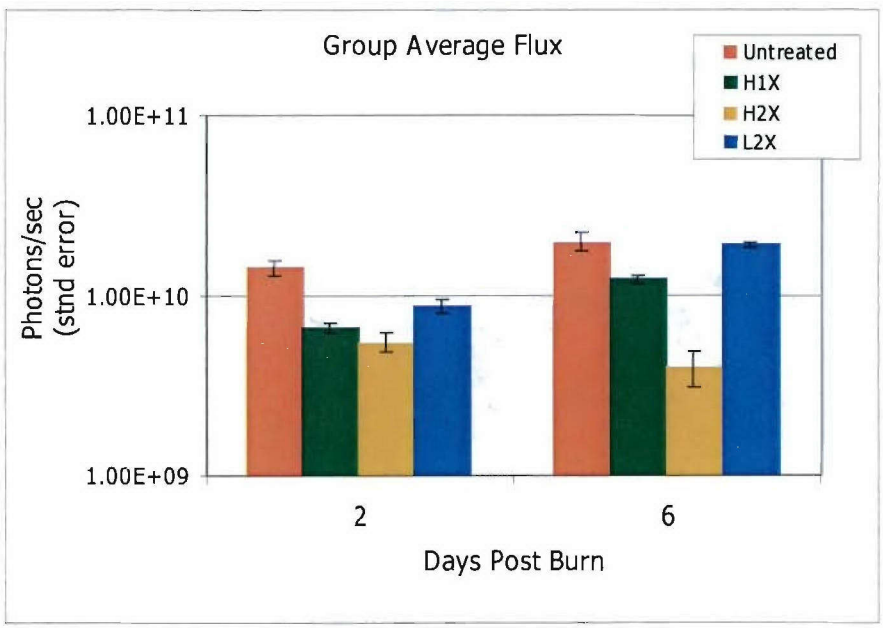


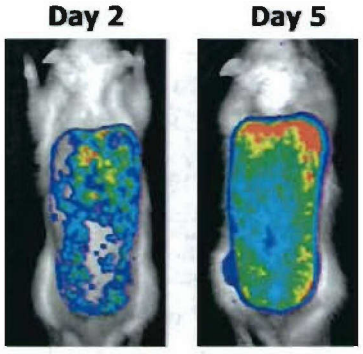
Figure 5. Average flux for each group on post burn day 2 and 6

Appendix A: Luciferase Imaging of Contamination and Infection
All animals are grouped by treatment, missing images indicate that no image was taken for that animal that day. For animals that were euthanized, the organs found to be infected with luminescent bacteria are listed.

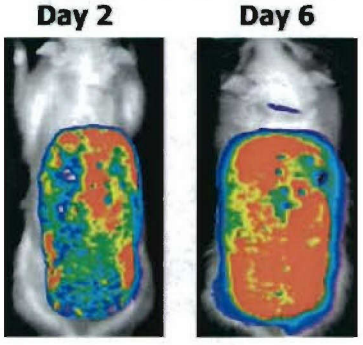
Silvadene

Silvadene treated positive controls

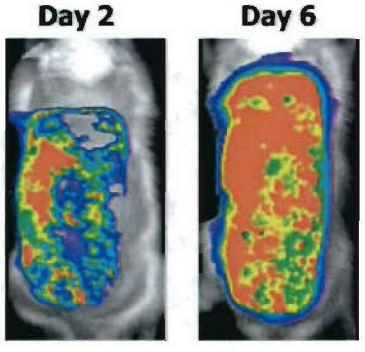
Untreated



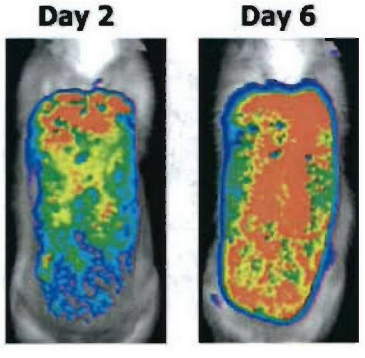
Day 5
Lum- Lunges, Spleen, Kidneys, Liver



Day 6
Lum- Lungs



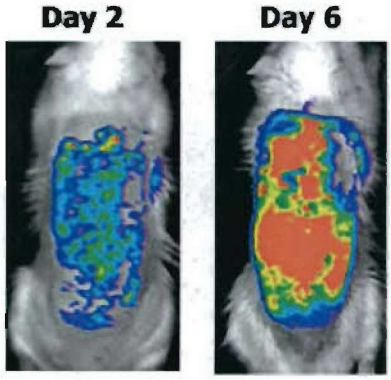
Day 6
Lum- Lungs, Spleen



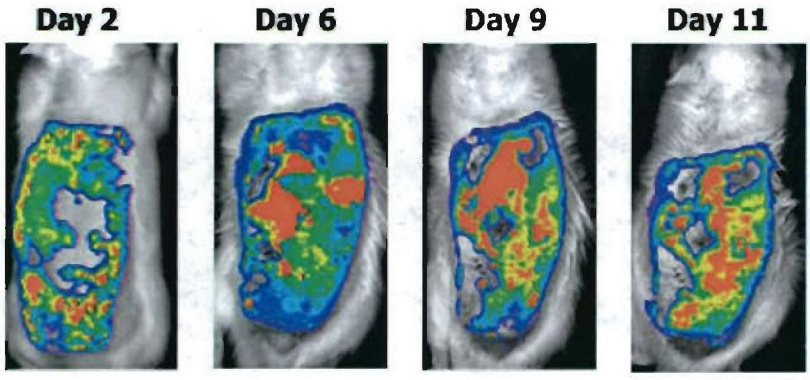
Day 6
Lum- Lungs, Spleen, Kidney

Untreated negative controls

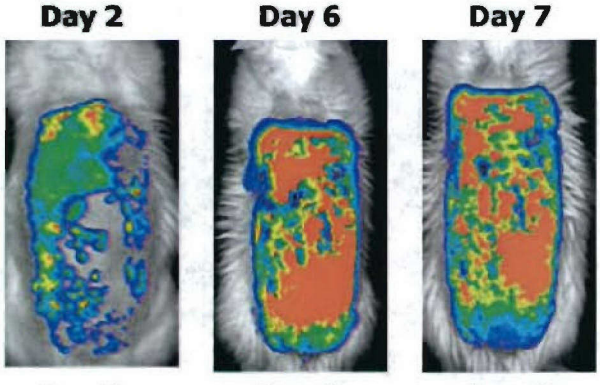
Low Concentration Twice Daily



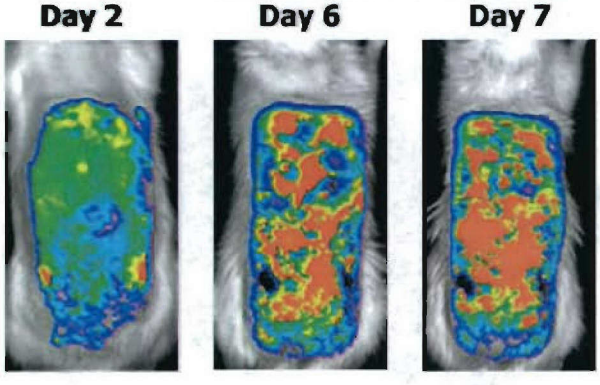
Day 6
Lum- Kidney



Day 11
Lum- Spleen



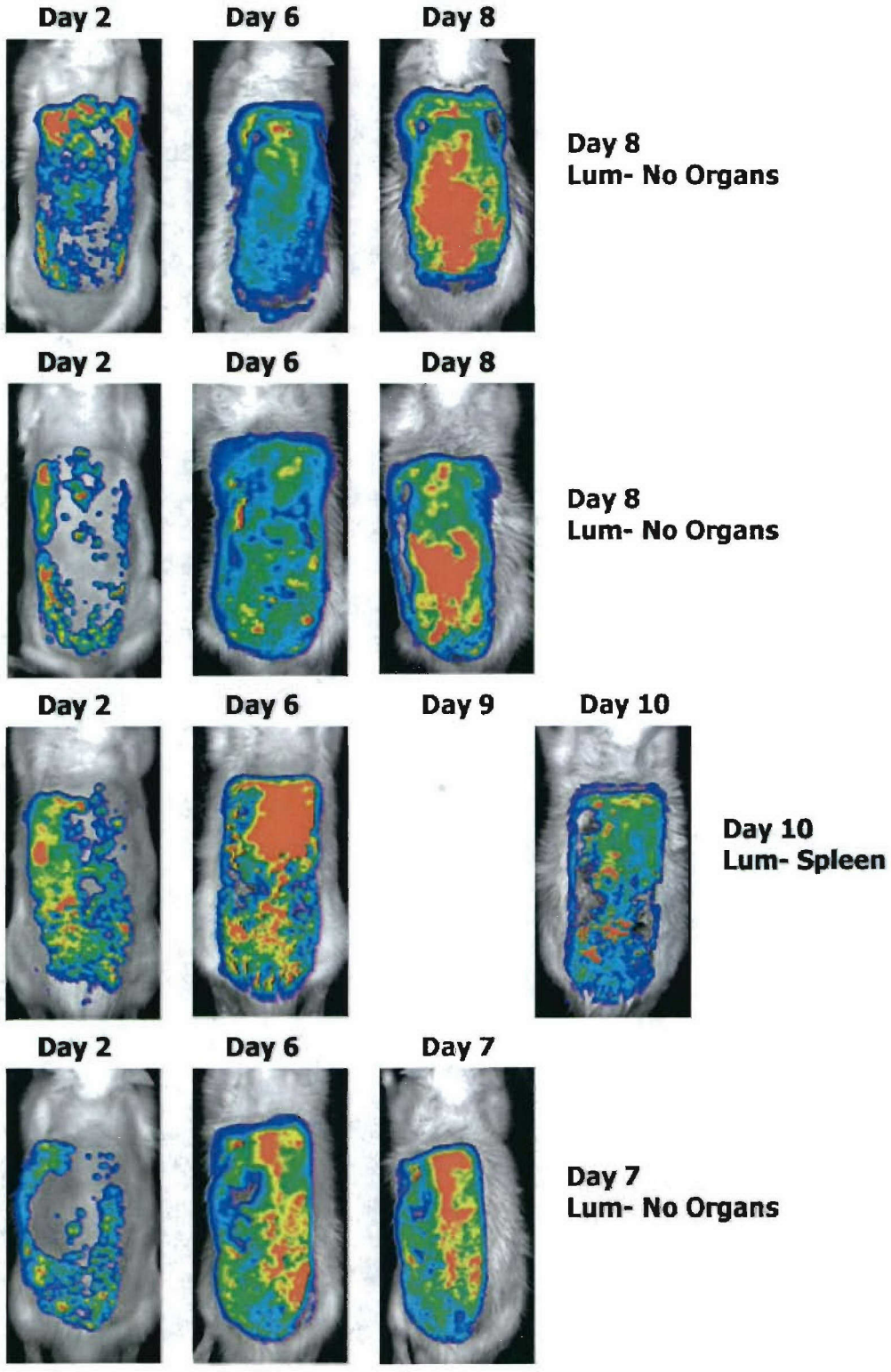
Day 7
Lum- Lungs, Liver, Spleen



Day 7
Lum- Kidney

Animals treated with low concentration solution twice a day

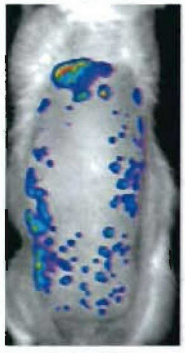
High Concentration Once Daily

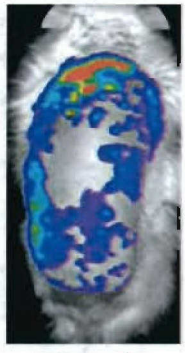


Animals treated with high concentration solution once a day

High Concentration twice daily

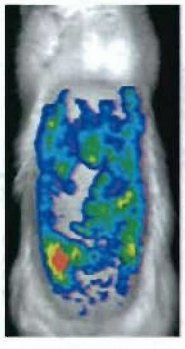
Day 2 **Day 4**

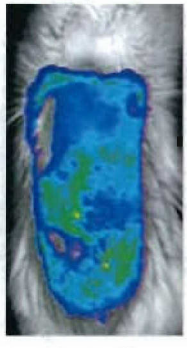


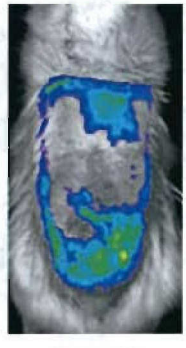


Day 4
Lum-Lungs, Liver, Spleen, Kidneys

Day 2 **Day 6** **Day 8**

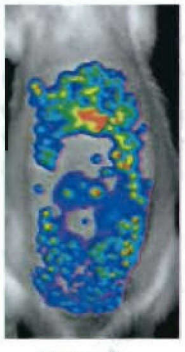


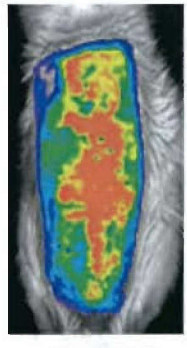




Day 8
Lum- No Organs

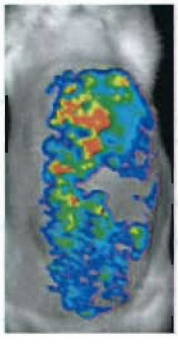
Day 2 **Day 6** **Day 9**

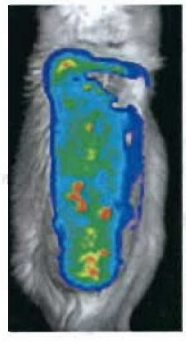




Day 9
Lum- Kidneys

Day 2 **Day 6** **Day 10**





Day 10
Lum -Lungs, Kidney, Liver

Animals treated with high concentration solution twice a day